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Morphologic Changes Following In Vitro CO_2 Laser Treatment of Calculus-Ladened Root Surfaces

Daniel Tucker, DDS, MS, Charles M. Cobb, DDS, PhD,
John W. Rapley, DDS, MS, and William J. Killoy, DDS, MS

Escondino, California (D.T.) and Department of Periodontics, School of Dentistry, University of Missouri-Kansas City, Kansas City, MO (C.M.C., J.W.R., W.J.K.)

Background and Objective: **The purpose of this study was to compare morphologic changes following CO_2 laser or manual curette treatment of calculus-ladened tooth root surfaces.**
Study Design/Materials and Methods: **Laser treatment consisted of repeated single passes with a 6 Watt focused beam at 20 pulses per second, a pulse length of 0.01 second, and a manufacturer's laser efficiency rating of 86% (i.e., the amount of total power delivered through the aperture). The rate of beam passage over the target surface was controlled at 4 mm/second using an 0.8 mm diameter tip. The calculated energy density was 240 J/cm^2 for each pass of the beam. Scaled and root planed surfaces were treated with a standardized force of 600 grams using new curettes. Specimens were evaluated by scanning electron microscopy.**
Results: **Laser-induced surface changes included charring, meltdown and resolidification of calculus mineral, and ablation of microbial plaque. Laser-treated specimens also exhibited residual calculus and microbial plaque deposits in areas directly adjacent to the beam path. Scaled and root planed surfaces featured smooth and/or scalelike smear layers and islands of residual calculus and microbial plaque.**
Conclusion: **The rough surface topography resulting from laser treatment and residual calculus and microbial plaque deposits indicates that CO_2 laser treatment of exposed root surfaces is, at best, an adjunct to traditional methods of therapy.**
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Key words: **bacteria, carbon dioxide laser, dental calculus, dental plaque, scanning electron microscopy, tooth root**

INTRODUCTION

The use of manual scalers for over a century and the use of both manual and ultrasonic scaling instruments during the last three decades have been the standard of care for tooth root debridement. However, the effectiveness of plaque, calculus, and absorbed endotoxin removal from root surfaces by manual or ultrasonic instrumentation, with or without surgical access, has limitations. Preliminary research has suggested that lasers of various wavelengths may have significant potential when used as an alternative or adjunctive means of removing microbial plaque and calculus from diseased tooth root surfaces.

Iwase et al. [1] used an He–Ne laser in a continuous mode to irradiate the gingival margins in hamsters to determine the inhibitory effect on plaque accumulation. Although the results were interesting and supportive of the laser, technical errors may have biased the results. Other investigators have reported both an immediate and an extended decrease in levels of microbial

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Address reprint requests to Dr. Charles M. Cobb, Dept. of Periodontics, School of Dentistry, Univ. of Missouri-Kansas City, 650 East 25th Street, Kansas City, MO 64108.

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pathogens after use of the Nd:YAG laser within periodontal pockets [2–4]. Wilder-Smith [5] used a He–Ne laser on human subjects to compare scaling and root planing with or without additional supragingival laser application. Sulcular bleeding index (SBI) measurements taken 1 month postoperatively did not differ between experimental and control, indicating no additional benefit from the laser. Frentzen and Koort [6] conducted an in vitro study using an ArF excimer (193 nm wavelength) laser to treat periodontally diseased roots. Their findings, using both light and electron microscopy, demonstrated that it is possible to remove plaque, calculus, and cementum by laser application without significant change in adjacent tissues. The extent of plaque, calculus, and root cementum ablation was directly dependent upon energy levels, length of exposure, and waveform (continuous, chopped, or pulsed beam). Their results demonstrated a smooth root surface topography, which is not typically found when using manual curettes or ultrasonic scalers. Tseng and Lew [7] have reported a separation between the calculus-root surface interface after using the Nd:YAG laser, which they claim facilitated calculus removal during subsequent manual instrumentation. Separations at dissimilar biologic interfaces may result from the "waveguide effect" described by Altshuler [8], i.e., a redirection and concentration of thermal energy along the interface.

A recent in vitro study has demonstrated that Nd:YAG laser-treated root surfaces are rendered incompatible to gingival fibroblast attachment [9]. This observation was confirmed in a subsequent study using the Nd:YAG laser at a decreased energy density [10]. However, after laser-treated surfaces were further treated by manual instrumentation, the surface became amenable to fibroblast attachment, suggesting that lasers may be used for root detoxification but only if followed by conventional scaling and root planing.

The mineral phase of dental calculus consists of calcium hydroxyapatite, octacalcium phosphate, brushite, and whitlockite [11]. Consequently, the characteristics inherent to the CO_2 laser wavelength, such as minimal depth of tissue penetration and maximum absorption by mineralized tissues due to nearly congruent wavelength and absorption spectra, indicate that the CO_2 laser might be a good choice for adjunctive use during tooth root detoxification. Thus the purpose of this study was to examine the morphologic effects of a CO_2 laser in vitro on dental calculus and microbial plaque versus that seen after removal using manual curettes.

MATERIALS AND METHODS

Specimen Preparation

The experimental specimens consisted of ten teeth extracted due to advanced periodontal disease. Teeth selected for the study met the following inclusion criteria: (1) visible calculus present on a flat proximal surface, (2) $>$ 5 mm of attachment loss, and (3) root surfaces free of gross caries or fractures.

All specimens were stored in 50 ml of sterile water containing 1,000 units/ml of penicillin, 100 µg/ml of streptomycin, and 2.5 µg/ml of amphotericin B. Selected teeth were randomly assigned to one of the two treatment groups, CO_2 laser or manual curette.

The treated surface, and therefore the evaluated field, encompassed a 5×5 mm (25 mm^2) area of proximal root. The specified surface area was outlined using a lead foil template that precisely defined the borders of the treated area, which in turn were easily identified during scanning electron microscopic (SEM) evaluation.

Laser Treatment

Laser treatment was accomplished using an LX-20 CO_2 laser (Luxar Corp., Bothell, WA) and consisted of repeated overlapping single passes (a total of ten passes) with a 6 Watt focused beam at 20 pulses/second, a pulse length of 0.01 second, and a manufacturer's laser efficiency rating of 86%, i.e., the amount of total power delivered through the aperture. The beam was delivered at a 90° angle to the surface and the rate of beam passage over the target surface was controlled at 4 mm/second using an 0.8 mm diameter ceramic delivery tip. The calculated energy density was 240 J/cm^2 for each pass of the beam (or a total energy density of 2400 J/cm^2 delivered to the 25 mm^2 surface area). The rate of exposure was standardized through use of a motorized microscope stage that moved the specimen at a predetermined rate under the laser handpiece, which in turn was held in a fixed position perpendicular to the specimen.

Treatment by Manual Curette

Specimens assigned to the manual curette treatment group were subjected to scaling and

root planing with new Columbia 4R/4L curettes using a standardized pressure of 600 grams. Selection of this scaling pressure was based on the observations of Bjorn and Lindhe [12], who determined 600 grams to be the average pressure used by clinicians in vivo. Standardization of pressure and curette to root surface angulation was accomplished by using the Nolte Pressure Maintenance Device (Dick Nolte, UMKC Dental School, Kansas City, MO). This device allows for a simulated, repeatable, root planing procedure to be performed using a constant instrument angulation and scaling pressure. The treated root surface area was subjected to 15 overlapping strokes.

Fig. 1. Cross-sectional view of dental plaque on an untreated portion of the root surface (arrow) illustrating density and thickness of mass (> 200 μm). Bar = 0.1 mm at an original magnification of ×221.

Scanning Electron Microscopy

Specimens were prepared for SEM evaluation by initial fixation in cold 2.5% glutaraldehyde in 0.1 mol/L cacodylate buffer at pH 7.4 for 4 hours. Following initial fixation, specimens were rinsed in buffer and dehydrated in a series of graded ethanol solutions (33–100%) at 45-minute intervals followed by immersion in hexamethyldisilazane for 4 hours. Specimens were then desiccated overnight, attached to aluminum mounts, and sputter-coated with 200 Angstroms of gold/palladium. All specimens were evaluated using a Phillips 515 SEM at 15 kV. Photographs were taken at positive angles of 15°, 45°, and 60° and at various magnifications.

RESULTS

SEM evaluation of manual curette-treated specimens was consistent with other instrumentation studies [13–15]. Noninstrumented areas adjacent to the treated surface exhibited undisturbed plaque composed of cocci, rods, and fusobacterium of various lengths, and thick layers of filamentous organisms (Figs. 1, 2). Such areas were characteristic of a dental plaque whose maturation process occurred by successional colonization and microbial coaggregation. Due to use of the template, distinct boundaries existed between the instrumented and noninstrumented areas (Fig. 3). The treated surface exhibited either a smooth and relatively clean smear layer (Fig. 4) or a scalelike smear layer comprised of residual debris derived from microbial plaque and calculus (Fig. 5). Shallow grooves and parallel instrumentation marks were characteristic of curette treated surfaces (Figs. 4, 5). Isolated islands of residual microbial plaque and calculus missed by the instrumentation process were also commonly observed (Fig. 5).

Laser-treated specimens exhibited a number of morphological changes associated with intense thermal energy. Surface areas within the direct path of the laser beam were characterized by complete ablation of the pervasive superficial layer of microbial plaque (Fig. 6). The peripheral zones of the beam path appeared to exhibit a thermal gradient in that one passed from a zone of obvious meltdown and resolidification and total ablation of microbes to an intermediate zone featuring a disturbed layer of plaque and calculus matrix to the most peripheral zone of undisturbed plaque (Fig. 7). Dental calculus exposed to the laser exhibited meltdown and resolidification and varying depths of vaporization or ablation (Fig. 8). The cooling and resolidification of the mineral phase of calculus resulted in a lavalike texture exhibiting an evenly distributed porosity (Fig. 9). The resolidified mineral phase often presented as a granular or globular texture with individual globules appearing to be loosely attached to the underlying surface (Fig. 9 insert). Unlike curette-treated specimens, the cementum surface of the root of those treated by laser was infrequently observed. This was due to the fact that the laser seldom removed the entire thickness of microbial plaque and/or calculus or generated a layer of residual, resolidified mineral presumably derived from calculus. In the few instances where the cementum surface could be inspected (Fig. 8), there

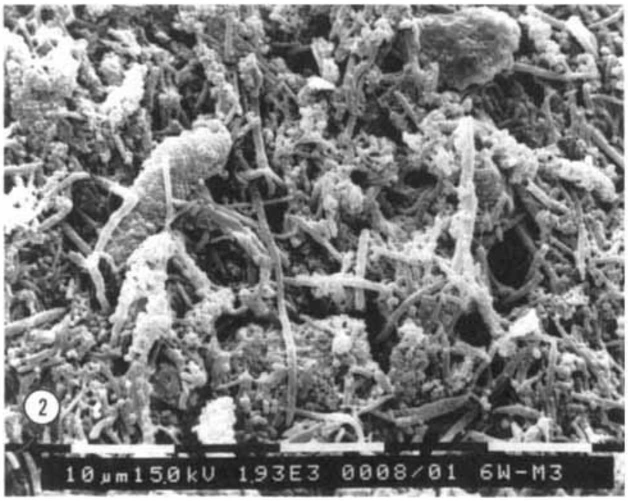

Fig. 2. View of a mature plaque mass on untreated portion of the root showing the diversity of morphotypes and evidence of microbial coaggregation. Bar = 10 µm at an original magnification of ×1,930.

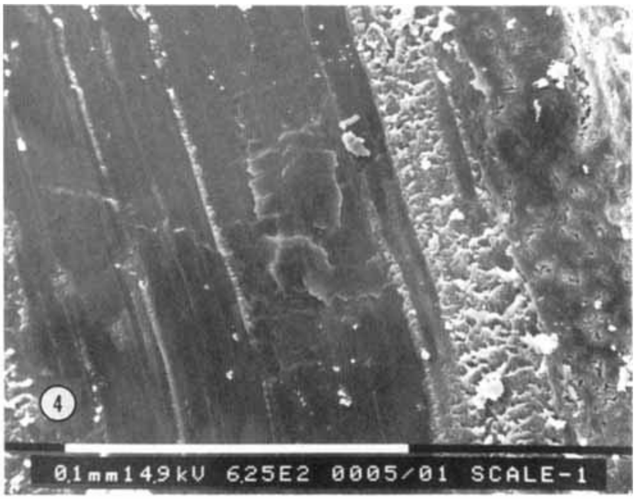

Fig. 4. Example of a relatively smooth root surface smear layer created by manual curette instrumentation. Untreated calculus is present at far right one-third of the photograph. Bar = 0.1 mm at an original magnification of ×625.

Fig. 3. Low angle view of root surface area treated by manual curette (scaling and root planing). Note the parallel instrumentation marks and ridge of surrounding untreated calculus. Bar = 1 mm at an original magnification of ×50.5.

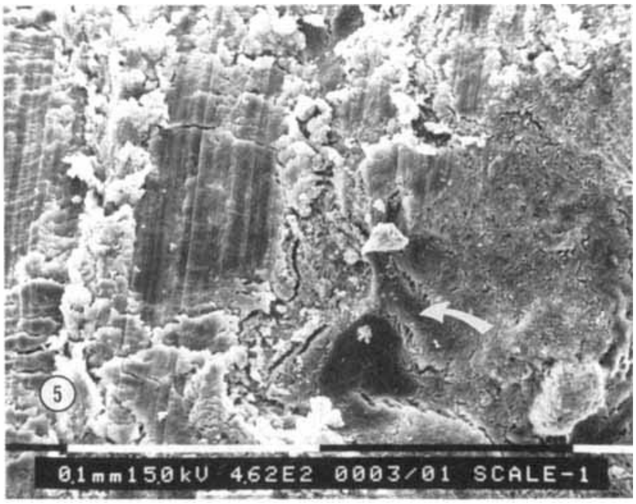

Fig. 5. Example of scalelike smear layer resulting from manual instrumentation of root surfaces. Residual calculus occupies the right one-half of photograph (arrow). Bar = 0.1 mm at an original magnification of ×462.

appeared to be little or no obvious surface damage. The penetration depth of laser-induced thermal damage was ~ 0.1 mm and often appeared to cause a separation of the root surface/calculus matrix interface (Fig. 10).

DISCUSSION

Under the conditions of this in vitro study, neither the CO_2 laser or the manual curette removed all root surface calculus and/or dental plaque. Rarely was the underlying cementum surface of the root exposed after curette or laser treatment. The use of manual curettes produced a surface smear layer comprised of calculus debris, microbes, and root cementum. In contrast, laser treatment vaporized microbial plaque deposits lying within the beam pathway, whereas calculus was transformed into deposits of residual mineral. At the energy density used in the present study, one must question the ability of lasers to remove dental calculus effectively, particularly

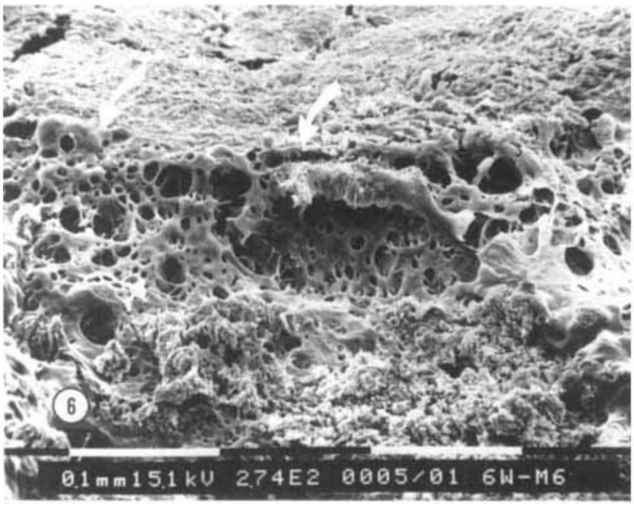

Fig. 6. Peripheral area of laser treated dental calculus demonstrating the abrupt transition (arrows) between treated and untreated surfaces. Note the granular and porous nature of the laser treated area (area below arrows). Bar = 0.1 mm at an original magnification of ×274.

Fig. 8. Low angle view of laser treated calculus showing incomplete removal and evidence of meltdown and resolidification of mineral phase. An undisturbed root cementum surface occupies the center of the photograph (arrow). Bar = 0.1 mm at an original magnification of ×178.

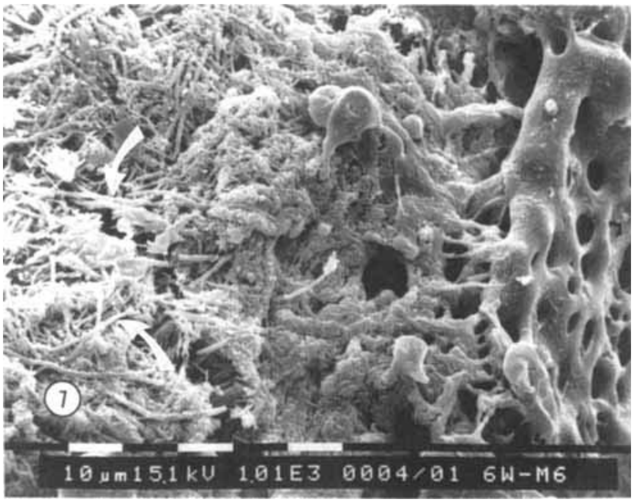

Fig. 7. High magnification view of the peripheral area of laser-treated dental calculus/microbial plaque. Laser exposure of calculus/plaque was exemplified by resolidified mineral and vaporization of microbes (right one-half of photograph). Microbes not in the direct beam pathway appear undisturbed (arrows). Bar = 10 μm at an original magnification of × 1,010.

since use of higher energy densities would increase the risk of collateral damage to adjacent tissues.

Tseng and Liew [7] used an Nd:YAG laser in vitro and demonstrated calculus meltdown similar to that observed in the present study. Furthermore, in both the present study and that of Tseng and Liew [7], separation of calculus from the subjacent root surface was noted. Tseng and Liew [7] also noted that when root planing with a manual curette, the number of required strokes to remove lased calculus and nonlased calculus was significantly different. Although the present study noted separations at the calculus/root surface and on occasion complete loss of the overlying plaque and calculus mass, it is difficult to ascribe the observation solely to effects of the laser. Dehydration of specimens in preparation for SEM examination may have contributed to the separation phenomenon due to differential contraction of tissues containing differing amounts of organic matrix and water. These observations suggest that lasers may be adjunctive rather than the primary method of calculus removal. Thus the problems of selecting appropriate wavelengths and parameters for the laser/root surface interaction remains a major obstacle to laser-mediated root therapy. Energy densities of sufficient magnitude to effectively ablate dental calculus, i.e., in excess of 240 J/cm^2 as used in the present study, would almost certainly result in significant heat damage to the tooth.

The CO_2 laser was clearly capable of eliminating the superficial microbial plaque layer that covers dental calculus. However, residual islands of plaque-covered calculus were left undisturbed due to the choice of pulsed mode beam delivery. The combination of pulsed mode (20 pulses per second) and rate of surface exposure to the beam

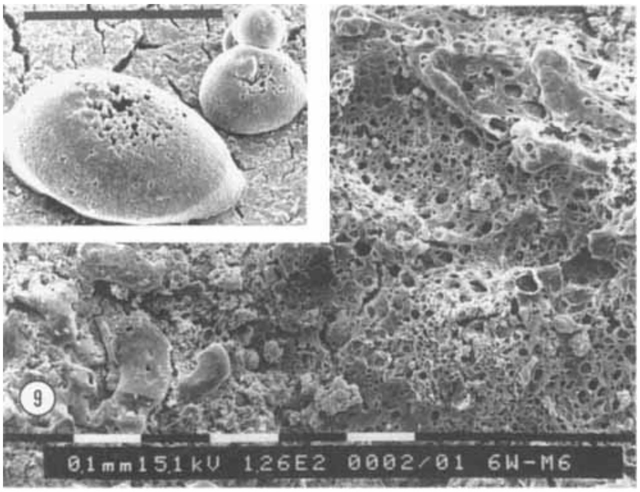

Fig. 9. View of laser-treated dental calculus showing the granular texture and porous surface texture. **Inset** is a globule of resolidified calculus mineral rather tenuously attached to the root surface. Bar = 0.1 mm at an original magnification of ×126; inset bar = 0.1 mm at a magnification of ×680.

Fig. 10. Low angle view of laser-treated calculus showing separation at the calculus/tooth surface interface (arrow). Separations of this type were not seen in untreated areas. Bar = 0.1 mm at an original magnification of ×775.

(4 mm/second) resulted in "skip" areas of residual microbial plaque. Although incomplete removal of microbial plaque may result in suppression of pathogenic flora below the threshold of disease, a reservoir of microbes persists that may facilitate a rapid repopulation of the pocket. Prevention of this phenomenon in vivo would require multiple overlapping passes of the pulsed beam or use of a continuous beam, thereby increasing the risk of collateral tissue damage. Further compounding of the problem is that the delivery system for CO_2 lasers prevents their use in periodontal pockets as the smallest commercially available delivery tip is metal encased and 0.35 mm in diameter. Even given adequate access, insertion of a rigid tip of this diameter into periodontal pockets would be difficult at best (particularly interproximal pockets) and limited to the first 2 or 3 mm. As most periodontal pockets requiring therapy vary in depths > 5 mm, the CO_2 laser can only be employed effectively for root therapy after surgical exposure of the root surface.

The estimated depth of laser energy penetration into the calculus/microbial plaque masses in this study approximated 100 μm. Microbial plaque in vivo frequently achieves a thickness exceeding 100 μm in just a few hours of active colonization [16,17]. Thus thick plaque masses, even after CO_2 laser irradiation, may retain viable microbes. Complete eradication of thickened calculus and plaque masses would require either a more penetrating wavelength, higher energy densities, or longer or repeated exposures—all of which increase the risk of incidental damage of adjacent tissues.

The process of phase conversion inherent to melting and resolidification of dental calculus resulted in a porous surface texture and an irregular topography comprised of what appeared to be loosely attached granules and/or globules of mineral. Although such areas were devoid of microbial plaque, one must question the role that roughened surfaces play in microbial recolonization and be concerned with the difficulty of maintaining such areas in a state of health. For example, Quirynen et al. [18] have evaluated the relative effects of surface free energy and surface texture on plaque formation and concluded that surface roughness had a greater influence on microbial accumulation. When the observations of the present study are combined with those of Quirynen et al. [18], one must assume that the persistence of laser-treated calcular debris requires removal by subsequent root planing with manual curettes or ultrasonic instruments.

In summary, the results of this in vitro study indicate that the CO_2 laser wavelength effectively vaporizes dental microbial plaque only within the beam path. Microbes lying peripheral to the beam path show little or no morphologic evidence of thermal damage. Dental calculus exposed to laser energy undergoes a phase transformation, i.e., meltdown followed by a resolidification during cooling. The results of this phase transformation

include a porous surface texture and a rough, irregular surface topography. An in vivo root surface of similar texture and contour would probably require root planing by manual instrumentation in order to achieve long-term maintenance goals. Consequently, the CO_2 laser appears best suited for tooth root therapy as an adjunct to periodontal surgery when there is complete exposure of the involved root surfaces.

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